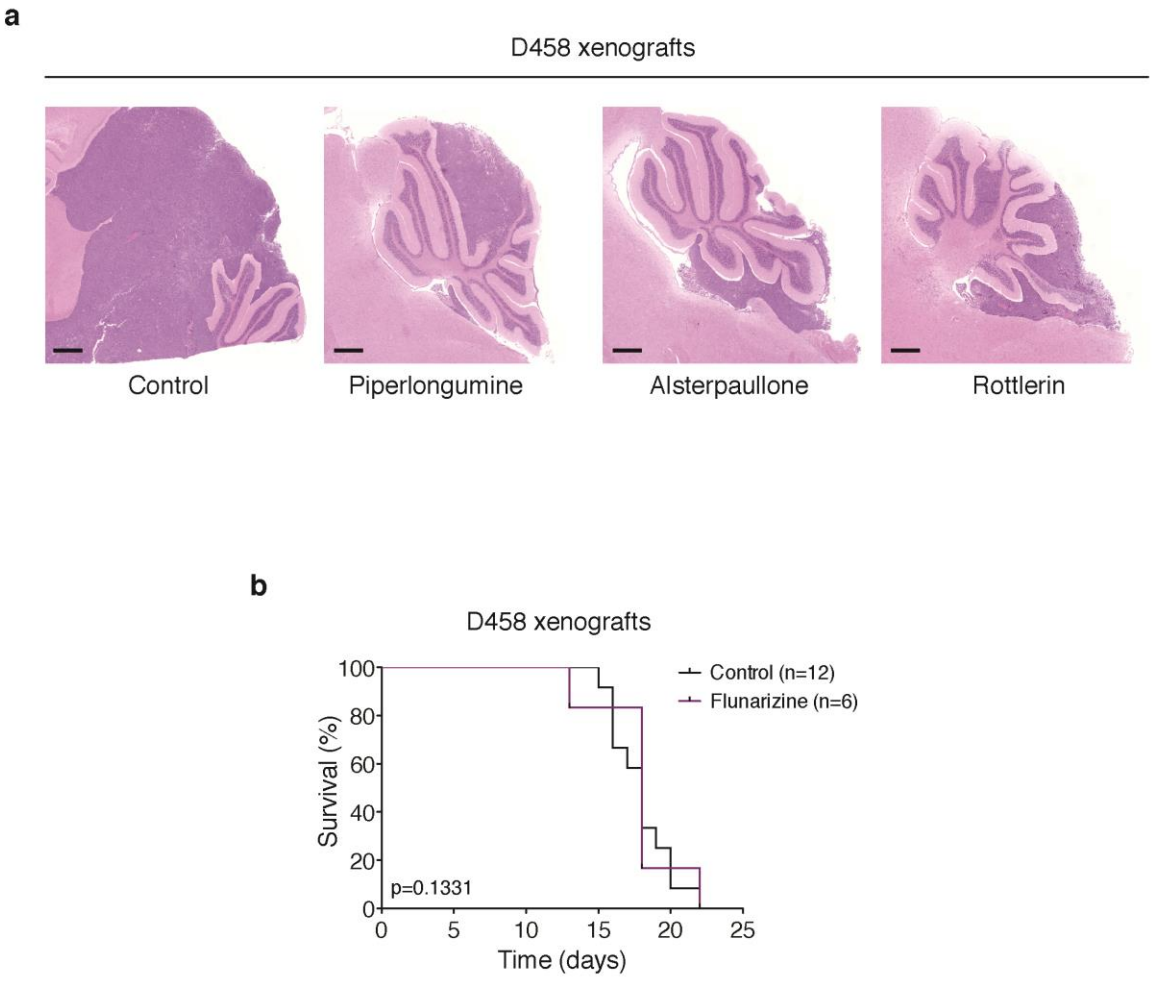
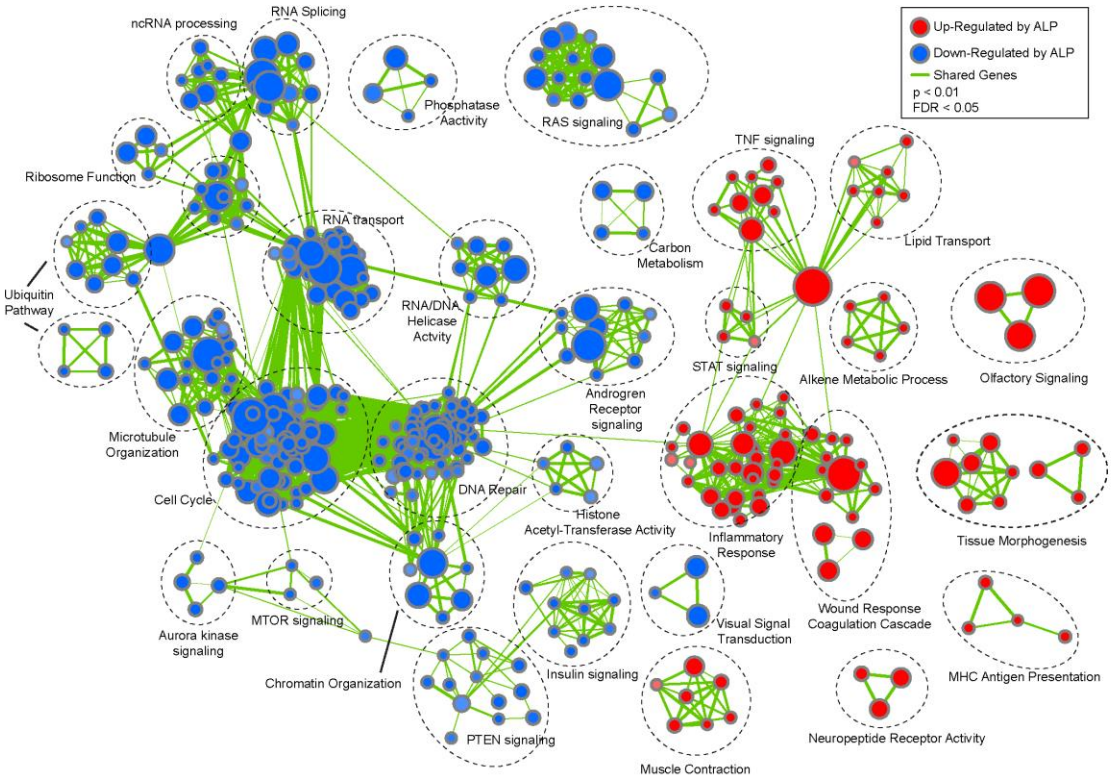
# Identification of alsterpaullone as a novel small molecule inhibitor to target group 3 medulloblastoma

**Supplementary Material**




**Supplementary Figure 1**. **a** Representative H&E staining demonstrates that mice bearing D458 tumors treated with piperlongumine, alsterpaullone and rottlerin have smaller medulloblastomas in the cerebellum. Scale bar: 500 µm. **b** Kaplan-Meier survival curves of D458 medulloblastoma xenografts treated with flunarizine (50 mg/Kg, daily for 2 weeks; n = 6) or vehicle control (10% DMSO; n = 12). Survival differences were calculated using a log-rank test.




**Supplementary Figure 2**. Biological pathways and processes up- and down-regulated by alsterpaullone. Gene Set Enrichment Analysis (GSEA) comparing gene sets up- and down-regulated by alsterpaullone (ALP) in D458 and D425 medulloblastoma cells (FDR < 0.05; p < 0.01). Cytoscape and Enrichment Map were used for visualization of the GSEA results. The enriched gene sets were grouped by their similarity, represented as nodes, and mapped as a network. The size of each node determines the total number of genes within each gene set.

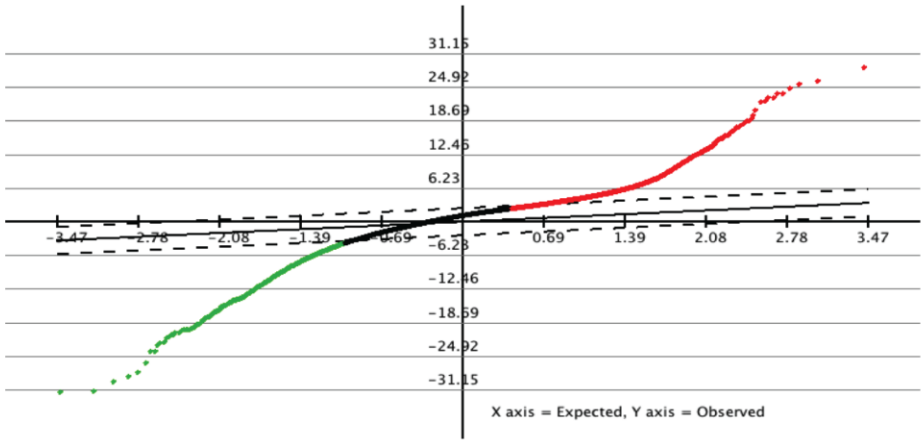
**a**

| cmap name | mean | n | enrichment | p-value | specificity | % non-null |
|---|---|---|---|---|---|---|
| alsterpaullone | 0.853 | 3 | 0.995 | < 0.000001 | 0.0116 | 100 |

**alsterpaullone**

| rank | batch | cmap name | | dose | cell | score | up | down | instance_id |
|---|---|---|---|---|---|---|---|---|---|
| 4 | 1066 | alsterpaullone | | 10 µM | MCF7 | .934 | .248 | -.366 | 7051 |
| 9 | 1067 | alsterpaullone | | 10 µM | PC3 | .904 | .208 | -.385 | 7056 |
| 31 | 1073 | alsterpaullone | | 10 µM | PC3 | .720 | .146 | -.327 | 7078 |

**b**




**Supplementary Figure 3**. Alsterpaullone reverses Group 3 medulloblastoma gene expression signature as shown by (a) the C-MAP analysis and (b) the Significance Analysis of Microarrays (SAM) plot.

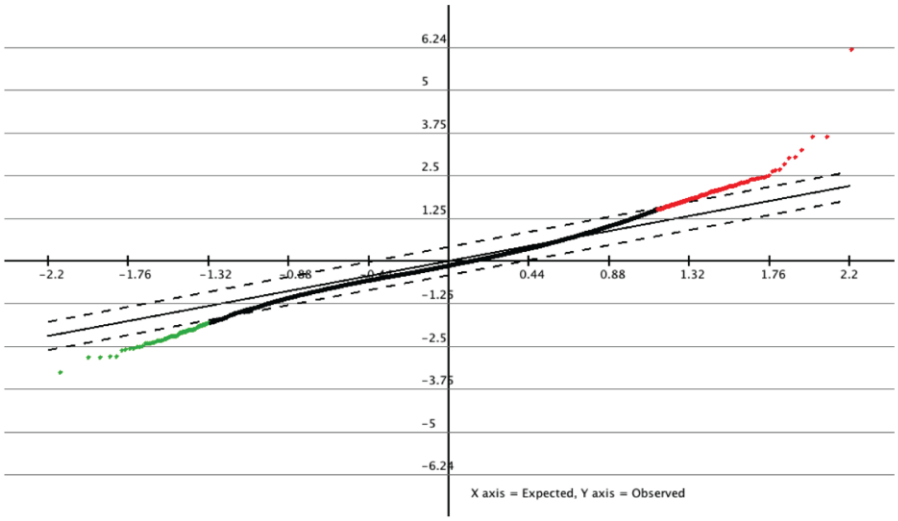
**a**

| cmap name | mean | n | enrichment | p-value | specificity | % non-null |
|---|---|---|---|---|---|---|
| piperlongumine | -0.061 | 2 | -0.474 | 0.59627 | 0.7342 | 50 |

**piperlongumine**

| rank | batch | cmap name | | dose | cell | score | up | down | instance_id |
|---|---|---|---|---|---|---|---|---|---|
| 614 | 641 | piperlongumine | | 13 µM | HL60 | .499 | .067 | -.095 | 1764 |
| 5943 | 662 | piperlongumine | | 13 µM | MCF7 | -.621 | -.128 | .130 | 2757 |

**b**




**Supplementary Figure 4**. The gene expression profile of Group 3 medulloblastomas is not affected by piperlongumine as determined by (a) the C-MAP analysis and (b) the Significance Analysis of Microarrays (SAM) plot.